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electrophysiology, we have found a hydrophobic barrier deep within the inner pore of the TWIK-1 channel [1]. Our study suggests that this barrier contributes to the very low level of functional currents observed for TWIK-1 channels. We have also reviewed the computational, structural and functional evidence for hydrophobic gating in several ion channel families and propose that understanding the dynamic behavior of water and ions within the pore represents an increasingly important element in understanding the relationship between ion channel structure and function [2]. We are now examining the interaction between K2P channels and phospholipids in more detail. Using MD simulations, we find hot spots for K2P channel and lipid interactions. These findings suggest that lipids can play modulatory roles in K2P channel function.

[1] Aryal P, Abd-Wahab F, Bucci G, Sansom MSP & Tucker SJ. A hydrophobic barrier deep within the inner pore of the TWIK-1 K2P potassium channel. Nature Communications 5:4377 (2014) [http://dx.doi.org/10.1038/ncomms5377]

[2] Aryal P, Sansom MSP and Tucker SJ. Hydrophobic Gating in Ion Channels. Journal of Molecular Biology (2014) [http://dx.doi.org/10.1016/j.jmb.2014.07.030]

2195-Pos Board B332

Non-Markovian Protein Dynamics in a Near-Critical Membrane Model

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Recent experiments in Giant Plasma Membrane Vesicles isolated from living cells have suggested that cell membranes are tuned close to a liquid-liquid critical point. Perturbations which influence membrane criticality - anesthetics and cholesterol level modulators - also affect the functioning of a large number of membrane-bound receptors and ion channels. This motivates us to develop a model for a membrane-bound protein that is allosterically regulated by interactions with its surrounding near-critical membrane. We consider a two dimensional lattice where Ising spins represent membrane lipids, and a two-state protein is represented as a group of like spins that must flip together. In our model, the full state of the system, including both protein and membrane degrees of freedom, obeys Markovian dynamics. However, when the protein is considered in isolation, as is typical experimentally, its dynamics become non-Markovian. We show that this phenomenon arises as information about the past state of the protein is stored in membrane degrees of freedom. Our model suggests a unified mechanism underlying the susceptibility of various ion channels to both anesthetics and cholesterol modulation and presents a new role for membrane lipids in the collective allosteric regulation of proteins.

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Mechanisms of TREK-2 Potassium Channel Gating

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The K2P channel TREK-2 is an archetypal polymodal potassium channel which acts to couple a diverse range of regulatory stimuli to cellular electrical excitability. Alongside the other thermo-and mechano-gated K2P channels (TREK-1 and TRAAK) the TREK-2 channel is critical for discrimination of innocuous and noxious temperature and touch sensation. Guided by novel X-ray crystal structures, we have used a variety of electrophysiological, pharmacological and kinetic studies to demonstrate a mechanism of action for the state-dependent inhibition of TREK-2 by norfluoxetine, a biologically active metabolite of the anti-depressant Prozac. These studies also enable us to propose a structural basis for activation of TREK-2 by membrane stretch, temperature and arachidonic acid.

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The Molecular Basis for Heme Modulation of KATP Channels

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Heme, iron protoporphyrin IX, is a vital prosthetic component of a number of functional hemoproteins playing essential roles in a diverse range of biological actions including oxygen transport, electron transport, catalysis and gene regulation. An emerging role of heme is its ability to regulate the activity of ion channels including voltage gated K^+ (Kv), large conductance Ca^{2+}-activated K^+ (BK) and epithelial Na^+ (ENaC) channels. Here we report heme regulation of the ATP-sensitive K^+ (KATP) channel in both cardiac myocytes and HEK293 cells heterologously expressing Kir6.2 and SUR2A subunits. The KATP channel is sensitive to the intracellular nucleotides ATP and ADP. The KATP channel links cellular metabolic state and excitability most notably during ischaemic stress. ATP acts with high affinity upon the cytosolic face to inhibit opening, thus the channel opens during periods of depleted cellular energy.

Whole-cell KATP currents of both ventricular myocytes and HEK293 cells expressing KATP channels were increased upon application of 500 nM hemin extracellularly. In inside-out patches, KATP channel activity was reduced in the presence of ATP (500 μM), but on subsequent addition of hemin (500 nM) KATP channel open probability significantly increased from 0.024 ± 0.013 to 0.110 ± 0.028 ($n = 6$, $P < 0.01$).

Sequence alignments with known heme binding regions revealed a structurally unresolved region located on an intracellular linker in the non-pore forming SUR2A subunit containing the residues C628XXH631 and H648, which was analogous to the reported Kv1.4 heme binding sequence. Mutating these residues (C628S, H631A and H648A) led to reduced sensitivity of the resulting KATP channels to heme. Mutagenesis of each residue revealed C628 and H648 to have the greatest effect at reducing the agonistic effects of heme. Here we provide evidence for heme binding and regulation of KATP

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NMR Structural Studies of the Binding of Activating Mamba Toxin Tx7335 on the Potassium Channel KcsA

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We have recently identified a novel 63 amino acid residue three-finger toxin (called Tx7335) from eastern green mamba snake (Dendroaspis angusticeps) which interacts with KcsA and induces an increase in frequency and duration of individual channel openings when added to the outside of the channel. The toxin exerts this activating effect both on wild-type KcsA as well as on an agitoxin2-sensitive mutant form of the channel, indicating a mode of action and binding site that are different from the classic pore-blocker toxins. We are currently using NMR spectroscopy to unravel the structural underpinnings of this mechanism of action. High yield of purified 15N labeled KcsA and excellent NMR spectral quality have been achieved. Currently the characterization of toxin binding using 1H15N correlation spectra of 15N labeled KcsA in the absence and presence of toxin is ongoing. Experiments are conducted in different membrane mimetics including DMPC/DHPC bicelles and DPC or DM micelles at different pH, temperature and salt concentration. Some chemical shifts and peak intensity changes upon toxin addition have been observed. Continuing NMR structural studies will further elucidate the mechanism of how Tx7335 interacts with KcsA and shed light on the conformational and dynamic changes of C-type inactivation in KcsA and on a novel mechanism of ion channel regulation.

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Mechanism of Inhibition of the GluA1 AMPA Receptor Channel Opening by 2,3-Benzodiazepine Compounds

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2,3-Benzodiazepine derivatives, also known as GYKI compounds, represent a group of the most promising synthetic inhibitors of AMPA receptors. Here we investigate the mechanism of inhibition of the GluA1 channel opening and the site of inhibition by GYKI 52466 and its N-3 methyl-carbamoyl derivative (BDZ-*f*) as well as two N-3 thiadiazolyl compounds. Like GluA2, GluA1 is a key AMPA receptor subunit, and excessive activity of GluA1 has been implicated in a number of neurological disorders. Using a laser-pulse photolysis technique, we investigated the mechanism of inhibition of the GluA1 channel expressed in HEK-293 cells. We found that these compounds inhibit the GluA1 channel noncompetitively. Addition of a methyl-carbamoyl group or a thiadiazole moiety to the N-3 position of the diazepine ring with the azomethine feature improves the potency of the resulting compounds without changing the site of binding, which we termed as the ''M'' site. On the basis of the magnitude of the inhibition constants for the same inhibitors (i.e., GYKI 52466 and BDZ-*f*), the ''M'' sites on GluA1 and GuA2 are different. Overall, the ''M'' site on GluA2 accommodates the same compounds better, or the same inhibitors show stronger potency on GluA2 than GluA1, if the N-3 pocket is not fully occupied. Acylating the N-3 position to occupy the N-3 side pocket of the ''M'' site can significantly narrow the difference and improve the potency of a resulting compound on GluA1. The two thiadiazolyl benzodiazepines inhibit both GluA1 and GluA2 much more strongly and almost equally potently. A thiadiazole moiety is thought to occupy more fully the side pocket of the ''M'' site, thereby generating a stronger, multivalent interaction between the inhibitor and the receptor binding site. *This work is supported by NIH/NINDS.*